Use of alprostadil (prostaglandin E1) for producing a medicament for angioneogenesis

The invention relates to the use of alprostadil for producing a medicament for angioneogenesis.

Cardiomyopathy (CMP) is a disease leading to myocardial enlargement and myocardial weakness. The myocardial weakness leads to a diminished pumping function and ejection action of the heart. The pathogenesis of dilative cardiomyopathy remains unknown. Ischemic cardiomyopathy may be attributable to myocardial infarction(s) because the dead areas of myocardium are replaced by connective tissue. However, this connective tissue replacement of myocardium is unable to carry out any cardiac functions. As a consequence, myocardial function is reduced and water accumulates in the lung and in the lower extremities. The results are severe dyspnea, incapability of exertion and tiredness.

The diagnosis of this disease consists of the typical signs and symptoms based on the myocardial weakness such as general weakness, incapability of physical exertion, dyspnea during exertion and at rest, recurrent pulmonary edema and deterioration in laboratory parameters with a pathological rise in kidney and liver parameters and a disturbed electrolyte balance. Echocardiography shows an enlarged heart with reduced ejection and an inhomogeneous contraction pattern. Patients with dilative CMP have no myocardial infarction in the history, and no (effective) coronary sclerosis is found in coronary angiography.

Alprostadil (PGE1) is a medicament which was originally used, because of its good vasodilating effects, in the treatment of neonates with (anatomical) cardiopulmonary malformations. A further limited use of alprostadil (PGE1) is chronic erectile dysfunction.

EP 0 153 858 A2 described the use of prostaglandins (including prostaglandin E1) for the treatment of multiple organ damage, acute respiratory distress syndrome (ARDS), shock, trauma or sepsis.

Forth et al. ("Allgemeine und spezielle Pharmakologie und Toxikologie", 7th edition (1996), page 344, column 1, 2nd paragraph) report exclusively the known vasodilating effect of prostaglandin E1 by describing the hemodynamic parameters for this indication which is the only one approved to date under the drugs legislation.

Rabinowitz et al. (Am. j. Ther. 4 (11/12) (1997), pp. 353-358) describe the hemodynamic effect of prostaglandin E1 on patients with coronary heart disease with stable and unstable angina pectoris, who have undergone an intervention with PTCA (heart catheter with balloon dilatation) or bypass operation or have suffered an acute myocardial infarction. A further finding is an increase in the skin temperature after use of prostaglandin E1 in patients with peripheral arterial occlusive disease.

Wimmer et al. (Jpn Heart J. 40(3) (1999), pp. 321-334) report the expression of neurohumoral mediators during prostaglandin E1 therapy compared with dobutamine in patients with chronic heart failure. This study shows a better hemodynamic effect in the group treated with prostaglandin E1 compared with the group treated with dobutamine. Renal function was also investigated by means of the paraaminohippurate clearance and the iothalamate clearance, and the patients in the prostaglandin group showed better kidney values.

In the article by Meyer et al. (Anesth. Analg. 86(1998), pp. 753-758), the hemodynamic effect of prostaglandin E1 in inhaled form combined with NO gas

was evaluated in patients with multiple organ failure, and these parameters showed an improvement with the form of therapy used.

Sterling et al. (Liv. Transp. Surg. 4(5) (1998), pp. 424-431) disclose the treatment of patients with acute hepatic failure, it being described as unclear whether prostaglandin E1 has any effect when this therapy is started within 10 days. However, it is pointed out that the results showed that prostaglandin E1 showed no effect when the treatment was not started until 10 days after the onset of the signs and symptoms. Accordingly, in the author's view, prostaglandin E1 is unsuitable for the treatment of acute hepatic failure (fulminant hepatic failure, (FHF)).

Finally, Iwata et al. (J. Gast. Hepatol. 14(1999), pp. 634-641) describe animal experiments in which prostaglandin E1 was infused directly into hepatic veins. The serology in this case showed more leukocytes in the group treated with prostaglandin E1. It is therefore concluded that hepatic perfusion with prostaglandin E1 microcirculatory damage associated with ischemia and reperfusion through inhibition of leukocyte-endothelium interactions can be treated.

It was an object of the present invention to provide a novel use of alprostadil (prostaglandin E1) which is not based on its vasodilating effect.

This object has been achieved by the use of alprostadil for producing a medicament for angioneogenesis. Further specific uses of the inventive angioneogenesis are described in claims 2-12. The effect, provided by the invention, of alprostadil on angioneogenesis was completely surprising in view of the known effects of prostaglandin E1. It has emerged that patients with chronic heart failure can be treated just as

successfully as patients with advanced peripheral arterial occlusive diseases, diabetic angiopathy, and systemic pulmonary disorders and acute or chronic renal or hepatic failure, and glomerulonephritis. It has also been possible further to revitalize dead areas of the heart, especially following a myocardial infarction. As a consequence, the inventive use is also possible in patients with cerebral infarction (infarction of the brain). This is attributable in any event to the fact that other functional processes than vasodilatation are responsible for this effect, e.g. a more efficient oxygen supply to the myocytes through an increased blood supply. Since this is dependent on the vessels and capillaries present, an increase in the blood supply after prostaglandin E1 infusion therapy would presuppose a neovascularization in the treated patients. It has also been possible to demonstrate this impressively by determining the vessel density within the scope of the present invention.

It is evident from the results described in detail in the examples that, surprisingly, neovascularization and thus an improvement in organ perfusion is present in the patients treated with alprostadil.

In contrast to the current doctrine, the alprostadil therapy used according to the invention evidently leads to:

a) new capillary formation and improvement in organ perfusion,

b) reduction in the pathological degree of fibrosis,

c) formation of tunnel capillaries,

d) regression of the muscle hypertrophy associated with chronic heart failure,

e) neovascularization, which is associated with increased VEGF production, with probable involvement therein also by other growth factors such as TFGB, PDGF, FGF;

f) increase in the cardiac index in cases of chronic

heart failure;

g) increase in the ejection action in cases of chronic heart failure;

h) mobilization of pulmonary edemas in cases of chronic heart failure;

i) reduction in the elevated pressures in the lesser circulation (pulmonary circulation) in cases of chronic heart failure;

j) stabilization of the blood pressure in cases of chronic heart failure;

k) improvement in the dyspnea and regression in the NYHA stage;

m) reduction in the muscle hypertrophy associated with CMP and hypertension-related cardiomyopathy.

PGE1 is preferably administered according to the invention by (intravenous) infusion. However, intracoronary administration may also be preferred, depending on the patient or pathological situation. Equally possibly indicatd is in particular epicardial administration in the pericardial cavity, local administration with the assistance of administration balloons, administration in coronary veins with the assistance of retrograde perfusion techniques, and transmyocardial administration with the assistance of laser, high-frequency ablation and/or injection needles.

The invention is explained in more detail by means of the following examples and the drawing figures, but it is not intended to be limited thereto.

These show:

Fig. 1 CD-34-positive capillaries in the subepicardium (Subepi), myocardium (Myocard) and subendocardium (Subendo) in alprostadil (PGE1)-treated patients compared with the control group;

Fig. 2 vWF-positive capillaries in the subepicardium (Subepi), myocardium (Myocard) and subendocardium (Subendo) in alprostadil (PGE1)-treated patients compared with the control group;

Fig. 3 MIB-1-positive endothelial cells in the subepicardium (Subepi), myocardium (Myocard) and subendocardium (Subendo) in alprostadil (PGE1)-treated patients compared with the control group;

Fig. 4 VEFG-positive capillaries in the subepicardium (Subepi), myocardium (Myocard) and subendocardium (Subendo) in alprostadil (PGE1)-treated patients compared with the control group;

Fig. 5 The degree of fibrosis in alprostadil (PGE1)-treated patients compared with the control group;

Fig. 6 Representative images of PGE1-treated patients (6A: CD-34-positive capillaries; 6B: vWF-positive capillaries, 6C: MIB-1-positive capillaries, 6d: VEGF-positive capillaries (the arrows mark positive capillaries in each case; Fig. 6A-C are taken with 1000× magnification; Fig. 6D with 400× magnification));

Fig. 7 A Sirius red stain (fibrosis content) of a patient after PGE1 therapy (Fig. 7A) and of a patient without PGE1 therapy (Fig. 7B);

Fig. 8 CD34 (treated (Fig. 8A) and untreated (Fig. 8B)), vWF (treated (Fig. 8C) and untreated (Fig. 8D), VEGF (treated (Fig. 8E) and untreated (Fig. 8F)) and MIB-1 (treated (Fig. 8G) and untreated (Fig. 8H)).

Examples:

1. Clinical study on patients with CMP

Patients with CMP were scheduled for a heart transplantation (HTX) and treated medically with ACE inhibitors, β blockers, diuretics and digitalis.

In a preceding study, several patients with cardiomyopathy, including 9 patients with dilative CMP, received alprostadil (PGE1) infusion therapy additionally before an HTX.

The criteria for inclusion in the alprostadil (PGE1) study were:

a. Patients were seriously restricted in their daily activity although they received a maximum oral medical therapy with ACE inhibitor (angiotensin converting enzyme antagonists), diuretics and digitalis.

b. The patients' hemodynamics showed a low cardiac index (< 2.5 1/min/m2) and a relatively high PCWP (pulmonary capillary wedge pressure) > 20 mm Hg).

c. CMP patients who showed an elevated peripheral vascular resistance.

Alprostadil (PGE1) does not form part of the standard therapy of cardiomyopathy, but has been used, because of its good vasodilating effect in patients with cardiomyopathy and elevated peripheral vascular resistance, as adjuvant therapy for relieving the stress on the heart until a heart transplant is performed.

The infusion therapy took place with continuous measurement of the hemodynamic parameters. A marked improvement in the hemodynamic parameters compared with the initial levels was evident with, at the same time, an improvement in the signs and symptoms. In this clinical experimental study, the alprostadil (PGE1) infusion therapy was used in patients in the terminal

stage of heart failure in the sense of an adjuvant therapy until the HTX. It is of interest that in this group of patients a marked improvement in the clinical condition with an increase in the ejection fraction and in the cardiac output was diagnosed, together with a reduction in the NYHA stage and in the pathological pressures in the pulmonary circulation.

The alprostadil (PGE1) infusions took place via a central right-heart catheter (Hickman catheter) with an initial dose of 2.5 ng/kg/min, which was then increased to the maximally tolerated dose (MTD). MTD was the dose at which one of the following side effects occurred: muscle pain, bone pain, fall in blood pressure, nausea, vomiting, diarrhea, headaches or other side effects. Below the MTD (29 ± 1 ng/kg/min), the hemodynamic parameters were recorded. The MTD was halved in the following 12 hours and, after the hemodynamics had stabilized, the therapy was continued with a portable pump at home.

In order to answer the question of whether alprostadil (PGE1) infusion therapy is associated with neovascularization, the HTX was followed by immunohistochemical investigation and comparison of the capillary density of the explanted hearts of patients with preceding alprostadil (PGE1) infusion therapy and of patients without alprostadil (PGE) therapy.

Each explanted heart was divided into three pieces of equal size, namely into the apex, middle and basal part. The cuts passed transmurally through the middle part of the left ventricle. The tissue for immunohistochemical analyses was preserved in formaldehyde by the usual method immediately after being removed.

The capillary density was determined separately in the subepicardium, myocardium and the subendocardium. The

capillary density/mm^2 was determined separately on the basis of anti-CD34, von Willebrand factor (vWf), vascular endothelial growth factor (VEGF), anti-Ki 67 (MIB 1) and Sirius red, immunohistochemically stained paraffin sections.

Results

The hemodynamic parameters before the alprostadil (PGE1) therapy were distinctly worse in the alprostadil (PGE1) group than in the control group, while the average age was comparable (52.33 ± 11.89 versus 49.88 ± 20.42 years, p = 0.76). The patients with alprostadil (PGE1) therapy had a significantly lower cardiac index (CI) (1.64 ± 0.29 L/min/m2 versus 2.39 ± 0.26 (L/min/m2, p <0.0001) and a higher pulmonary vascular resistance index (PVRI) (605.11 ± 149.80 dyn × sec × cm-5 × m-2 versus 372.75 ± 84.63 dyn × sec × cm-5 × m-2, p = 0.0015). Both groups had a comparable medical treatment apart from the alprostadil (PGE1) therapy.

The capillary density in the subepicardium, myocardium and subendocardium of transmural sections was determined quantitatively and compared. Patients who received alprostadil (PGE1) infusions had distinctly more capillaries (p <0.001) per mm^2 than the control group. The capillary density in the subepicardium, myocardium and subendocardium of the control group was 608.56 ± 91.32 capillaries/mm^2 (sEpi), 542.44 ± 197.20 capillaries/mm^2 (sEndo), and 452.22 ~ 101.99 capillaries/mm^2 (Myo) in the three transmural areas. Alprostadil (PGE1)-treated patients had by comparison therewith approximately 50% more capillaries than the control group, with 1168.11 ± 165.04 capillaries/mm^2 (sEpi), 1066.00 ± 94.63 capillaries/mm^2 (SEndo), and 974.56 ± 87.12 capillaries/mm^2 (Myocard).

Investigation of specific immunohistochemical capillary

markers:

CD 34, endothelial cell marker

CD 34 is expressed by all endothelial cells in normal tissue. CD 34 is also used for detecting endothelium and endothelial cells. Patients after an alprostadil (PGE1) therapy had distinctly more anti-CD34-reactive endothelium (Fig. 1) than the control group (subepicardium: 599.22 ± 107.17 mm^2 versus 322.89 ± 160.64 mm^2 cells, $p < 0.001$; myocardium: 482.11 ± 79.86 mm^2 vs. 227.22 ± 49.30 mm^2, $p < 0.0001$; subendocardium: 482.11 ± 79.86 mm^2 versus 227.22 ± 49.30 mm^2, $p < 0.0001$); subendocardium: 551.67 ± 107.74 mm^2 versus 308.56 ± 193.86 mm^2, $p < 0.01$). The results are also depicted in fig. 6A, 8A and 8B.

Factor VIII-related antigen, von Willebrand factor (vWf)

Factor VIII was investigated immunohistochemically as second pan-endothelial cell-specific marker. The hearts of CMP patients after alprostadil (PGE1) infusion therapy showed significantly more anti-vWf-reactive endothelia (fig. 2) compared with the control group (subepicardium: 425.56 ± 134.17 mm^2 versus 192.22 ± 77.88 mm^2 cells, $p < 0.001$; myocardium: 360.00 ± 52.31 mm^2 versus 159.89 ± 61.00 mm^2 $p < 0.0001$; subendocardium: 408.00 ± 80.00 mm^2 versus 163.89 ± 47.52 mm^2, $p < 0.0001$). The results are also depicted in fig. 6B, 8C and 8D.

MIB-1 (proliferation marker)

MIB-1 (anti-Ki67) is an antibody which reacts only with cells which are not in the GO phase of the cell cycle and is normally employed as marker of mitosis and proliferation. It shows a characteristic reaction with mitotic cells.

The number of MIB-1-positive cells (fig. 3) in all layers of the myocardium was distinctly higher in the

group of patients with alprostadil (PGE1) infusion therapy compared with the control group: (subepicardium: 20.22 ± 4.87 per mm^2 versus 8,11 ± 2.67 per mm^2, p <0.0001; myocardium: 15.44 ± 4.64 per mm^2 versus 5.78 ± 2.11 per mm^2, p <0.0001; subendocardium: 17.84 ± 4.23 per mm^2 versus 6.89 ± 2.21 per mm^2). The results are also depicted in fig. 6C, 8G and 8H.

Anti-VEGF immunoreactivity of capillaries

VEGF is described as specific growth factor for endothelial cells. Alprostadil (PGE1)-treated patients showed significantly more VEGF-positive capillaries/mm^2 than the control group (fig. 4) in all three investigated planes of section (subepicardium: 101.2 ± 5.5/mm^2 versus 38.1 ± 7.2 VEGF-positive cells/mm^2, p <0.0001; myocardium 76.2 ± 4.9/mm^2 versus 20.6 ± 4.9 mm^2, p <0.0001; subendocardium: 89.1 ± 5.7/mm^2 versus 27.8 ± 5.1/mm^2,, p <0.0001). The results are also depicted in fig. 6D, 8E and 8F.

It is thus proved that - in contrast to the conventional doctrine - alprostadil (PGE1) therapy leads to neovascularization of the heart in patients with CMP. These properties are in favor of alprostadil (PGE1) therapy as standard therapy for patients with chronic hear failure and cardiomyopathy.

Fibrosis and muscle mass (Sirius red stain)

Determination of the degree of fibrosis and of the proportion of myocardium in alprostadil (PGE1)-treated patients (fig. 5) compared with the control group showed that the alprostadil (PGE1) infusion therapy distinctly reduces the degree of fibrosis in patients with dilative CMP (15.35 ± 10.32% versus 6.89 ± 3.59%, p <0.05, with a comparable proportion of myocardium (72.69 ± 5.25% versus 68.76 ± 6.23%).

Hypertrophy:

The muscle number/mm^2 with percentage muscle

proportion/mm^2 were determined in PGE1-treated patients and compared with untreated patients and healthy controls. The results are shown in the table below.

	Muscle number/mm^2	% Muscle proportion/mm^2
PGE1-treated patients	982.68 ± 141.43	74.9 ± 3.7
Untreated patients	865.35 ± 160.64	69.7 ± 2.8
Controls	1265.04 ± 59.82	79.5 ± 2.5